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CANADIAN THESES ON MICROFICHE | THÈSES CANADIENNES SUR MICROFICHE

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TITLE OF THESIS/*TITRE DE LA THÈSE* Geographical and altitudinal variation in enzyme polymorphism and colour among western populations of Elaphrus americanus Dejean (Coleoptera: Carabidae: Elaphrini)

UNIVERSITY/*UNIVERSITÉ* Univ. of Alberta

DEGREE FOR WHICH THESIS WAS PRESENTED/ *GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* MSc

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE GRADE* 1978

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GEOGRAPHICAL AND ALTITUDINAL VARIATION IN ENZYME POLYMORPHISM AND COLOUR AMONG WESTERN POPULATIONS OF *ELAPHRUS AMERICANUS* DEJEAN (COLEOPTERA: CARABIDAE: ELAPHRINI)

by

© HENRY E. FRANIA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

ENTOMOLOGY

EDMONTON, ALBERTA

FALL 1978

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled GEOGRAPHICAL AND ALTITUDINAL VARIATION IN ENZYME POLYMORPHISM AND COLOUR AMONG WESTERN POPULATIONS OF ELAPHRUS AMERICANUS DEJEAN (COLEOPTERA: CARABIDAE: ELAPHRINI)
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Abstract

Samples of the ground beetle species Elaphrus americanus Dejean, from subalpine areas in the Pacific Northwest, were compared to collections taken at lower elevations and were found to differ in the habitats where beetles could be taken, in the frequency of electrophoretic variants at an esterase and an aldehyde oxidase locus, and in frequencies of several body colour morphs. The distinctive subalpine populations were found on Tusk Mountain, British Columbia; Mount Baker and Mount Rainier, Washington. All samples up to an elevation of 1000 m on these mountains and at all elevations on Mount Adams, Washington and Mount Hood, Oregon, had similar frequencies of electrophoretic variants. In these populations, the level of esterase variation was comparable to that of the most polymorphic species known. Above 1000 m, enzyme variation was reduced. The three subalpine populations differed from one another in esterase allele frequencies and, a colour morph not found elsewhere, was common in the subalpine sample from Mount Rainier. The patterns of variation were stable over time and independent of adult age. Transition from the low altitude to the subalpine populations was abrupt, occurring over a maximum altitudinal distance of 600 m on Mount Rainier. The origin of subalpine populations by one or more founder events is unlikely since the beetles fly well and disperse extensively and, there is indirect evidence for multiple dispersals into subalpine areas. Subalpine populations probably

differentiated *in situ* from the low altitude populations, since the sharp break between the two forms corresponds to an equally abrupt change in environmental conditions with elevation which (1) limits movement of individuals between low and high altitude populations because, at high elevations, heavy snow accumulation retards onset of the summer season, and (2) results in differential selection pressures which are probably strong enough to offset the effect of any gene flow between the populations. Recent adjustments of populations to local conditions at higher altitudes is probable since a small sample in the transition zone on Mount Rainier showed esterase allele frequencies characteristic of the low altitude population, but had a subalpine pattern of aldehyde oxidase variation. Subalpine populations may have differentiated to the species level, because these occur in specialized subalpine habitats not found at lower elevations, but evidence from data on enzyme and colour variation is equivocal. Mount Adams and Mount Hood are thought to be too dry to support permanent high altitude populations.

ACKNOWLEDGMENTS

I thank my sponsor, G. E. Ball for his support and advice during this study; B. B. Chiolino for suggesting a study of this nature, and for his encouragement in the initial stages of the work; H. Goulet for pointing out the unusual nature of subalpine populations of *Elaphrus americanus*, and for much information on the identification, collection and natural history of these beetles; R. H. Gooding for access to his laboratory facilities and for advice; B. M. Rolseth for considerable help with electrophoretic techniques; M. McIntyre for assistance in fabrication of equipment; J. S. Scott for assistance with graphical layout and lettering of figures.

I thank H. C. Wills, chief naturalist, Mount Rainier National Park, for park collecting permits.

I thank the following people for reviewing the manuscript: G. E. Ball, R. H. Gooding, K. E. Denford (Dept. of Botany), and J. Belicek.

I thank J. Houseman and D. Reinhart (Computing Services) for assistance in computer production of this thesis.

The support, tolerance and assistance of my fellow graduate students during this study, is appreciated.

I acknowledge financial support for this work from National Research Council of Canada grant A1399, awarded to G. E. Ball.

Table of Contents

1. Introduction.....1

   1.1 Definition of general problem.....1

   1.2 Natural history and population structure of *Elaphrus americanus*.....2

   1.3 Definition of specific problems.....3

2. Materials and Methods.....4

   2.1 Materials and collecting sites.....4

   2.2 Methods.....6

      2.2.1 Electrophoretic variation.....6

      2.2.2 Colour variation.....8

      2.2.3 Age of adults.....9

   2.3 Statistical analyses.....10

3. Results.....12

   3.1 Habitat comparisons between subalpine and lower altitude populations.....12

   3.2 Life cycle comparisons.....14

   3.3 Interpretation of enzyme variation.....15

   3.4 Patterns of enzyme variation.....16

      3.4.1 Low and mid-elevations populations.....16

      3.4.2 Subalpine parkland samples.....18

      3.4.3 Subalpine forest samples.....20

      3.4.4 Patterns of enzyme variation-summary.....21

   3.5 Patterns of colour variation.....21

4. Discussion.....23

   4.1 Genetic interpretation of enzyme and colour polymorphism data.....23

   4.2 Origin of subalpine populations: Possibilities.....25

4.3 Primary intergradation: evidence.....26

4.3.1 Environmental gradients.....26

4.3.2 Role of selection.....27

4.3.3 Role of gene flow.....30

4.4 Secondary intergradation.....33

4.5 Reproductive relationships.....36

4.5.1 Populations on a mountain.....36

4.5.2 Subalpine populations on different mountains.....37

4.6 General considerations.....38

4.6.1 Prevalence of microgeographic races.....38

4.6.2 Microgeographic races: evolutionary potential.....39

4.6.3 Microgeographic races: taxonomic implications.....41

4.7 Concluding remarks.....42

References.....46

Vita.....53

LIST OF TABLES

Table . . . . . Page

Table 1. Classification of variation of EST-1 in samples of *Elaphrus americanus* from the Pacific Northwest.....55

Table 2. Classification of variation of AO-1 in samples of *Elaphrus americanus* from the Pacific Northwest.....56

Table 3. Variation in occurrence of eggs in females and in sclerotization of the aedeagus of males for samples of *Elaphrus americanus* collected on Mt. Rainier, Washington in July, 1976; August, 1977.....57

Table 4. Comparison of observed and expected number of individuals under assumption of Hardy-Weinberg equilibrium for commonest EST-1 phenotypes in samples of *Elaphrus americanus* from the Pacific Northwest.....58

Table 5. Comparison of observed and expected number of individuals under assumption of Hardy-Weinberg equilibrium for commonest AO-1 alleles in samples of *Elaphrus americanus* from the Pacific Northwest.....59

Table 6. Comparison, by Spearman's rank correlation test of EST-1 and AO-1 allele frequencies between selected samples of *Elaphrus americanus*.....60

Table 7. Relationship of small samples to larger samples of *Elaphrus americanus* from the Pacific Northwest showing the probability of obtaining the actually observed number of individuals with a particular phenotype if that sample had been taken from an adjacent larger sample.....61

Table 8. Comparision, by Spearman's rank correlation test of the distribution of EST-1 and AO-1 allozymes with respect to colour morphs in selected samples of Elaphrus americanus.....62
Table 9. Colour morph frequencies in samples of Elaphrus americanus from the Pacific Northwest.....63

LIST OF FIGURES

Figure Page

Figure 1. Map showing the relationship of subalpine forest, alpine areas, extent of Cordilleran glaciation in the Pleistocene and collecting sites of *Elaphrus americanus*.....64

Figure 2. Dorsal view of an adult male showing principal features which determine overall body colour.....65

Figure 3. Polyacrylamide gel showing banding patterns of a monomeric esterase (EST-1) from individuals of *Elaphrus americanus* from various localities in the Pacific Northwest.....66

Figure 4. Polyacrylamide gel showing banding patterns of a dimeric aldehyde oxidase enzyme (AO-1) from individuals of *Elaphrus americanus* from various localities in the Pacific Northwest.....66

Figure 5. Frequency distributions of EST-1 alleles from samples of *Elaphrus americanus* from four mountains....67

Figure 6. Frequency distributions of AO-1 alleles from samples of *Elaphrus americanus* from four mountains....68

1. Introduction

1.1 Definition of general problem

Mountains have figured prominently in the study of evolutionary processes. Studies of population variation with altitude on mountains were early demonstrations of the degree to which organisms may adapt to local environments (Clausen, Keck and Heisey, 1940), and also indicated that strong selection pressures can exist in nature (Dobzhansky, 1948). Workers interested in the speciation process (J. M. Diamond, cited in MacArthur, 1972) and in the historical origin of biotas (Darlington, 1971) have used to advantage the fact that environmental gradients are repeated over and over on the various peaks of a mountain range, and that altitude affects the distribution and makeup of populations of most species present on those mountains.

One consideration of general interest is the conditions under which populations of a given species adapt to new environments. The most favourable situation is thought to be at the distributional limits of a species, where environmental conditions are often marginal and the populations are small and isolated (Carson, 1959; Mayr, 1963). Such species distributions not only have geographical boundaries, but also altitudinal limits. This study was an attempt to determine how, a widespread species of ground beetle Elaphrus americanus Dejean (Carabidae), apparently

extended its upper altitudinal limit in the mountains of the Pacific Northwest to include a habitat type not found at lower elevations.

1.2 Natural history and population structure of *Elaphrus americanus*

Elaphrus americanus is found throughout forested regions across Canada and the northern United States (Lindroth, 1961). At low elevations, adults and larvae live at the edges of ponds or slow-moving streams on recently exposed, bare, wet, mud or clay flats. Adults are predacious and hunt on the surface of the mud during the day under bright or sunny conditions, and probably as a consequence, they are cryptic in behaviour and appearance. Individuals live one year, overwinter as adults, and then reproduce for several months (spring to mid-summer) and mate often. Habitats are scattered and there is probably considerable dispersal of adults between sites, as these beetles are strong fliers and the habitat sites tends to dry up, flood, or grow over with vegetation (pers. obs.). Thus, it is likely that populations are panmictic over large areas.

1.3 Definition of specific problems

In the course of a generic revision of Elaphrus, my colleague, Henri Goulet (pers. comm.), collected hundreds of adults of E. americanus near the edges of melting snowbanks in subalpine areas of the Cascade and Coast Mountains of the Pacific Northwest. Goulet found that subalpine samples differed from low altitude samples in means of several character measurements and in frequencies of some colour morphs.

It seemed reasonable to assume that E. americanus adapted secondarily to subalpine habitats. Subalpine populations, although peripheral in distribution, could hardly be considered isolated from lower altitude populations given the dispersal capabilty of this species and it was therefore of interest to determine what were the relationships between high and low altitude populations. Three specific questions were asked. Are the subalpine and low altitude populations genetically differentiated? If so, are the populations genetically continuous with one another at mid-elevations? The mid-elevation samples should then be intermediate in various characteristics. Finally, are the subalpine populations derived independently on some mountains?

2. Materials and Methods

2.1 Materials and collecting sites

Samples were collected along several mountain transects (Fig. 1) in July, 1976 and August, 1977, from low elevations (<300 m), mid elevations (<1000 m) and from higher elevations in the subalpine zone (as defined by Krajina, 1970). Each transect was centered on a mountain from which a high altitude population of E. americanus was previously known. Samples were taken to encompass a spectrum of environmental conditions present on the West Coast, e.g., a transect across Mt. Rainier encompassing wet coastal forest in the west (Brooklyn Road) and dry ponderosa pine forest in the east (Nile Creek). Samples were also taken to assess the effect of potential dispersal barriers (the Cascade Crest and the Columbia River basin) on the makeup of several populations of this species. The transects are rather widely spaced apart, but additional collections were not made because of time limitations. In total, 706 specimens were examined from 17 samples (sample sizes given in Tables 1 and 2).

LOCALITIES:

British Columbia: Tusk Mtn. transect. MIMULUS LAKE, Garibaldi Prov. Park 49°57'55" 123°01'55", 1722 m, 1 SEP 1977.

Washington: Mt. Baker transect. ANDERSON CREEK, Mt. Baker

Snoqualmie Nat'l. Forest 48°54'00" 121°43'10", 610 m, 30 AUG 1977; BAGLEY LAKES, Mt. Baker-Snoqualmie Nat'l. Forest 48°51'25" 121°41'20", 1290 m, 30 AUG 1977. Mt. Rainier transect. BROOKLYN ROAD (Butte Creek), 1 mi. n. of Raymond 46°43'00" 123°43'50", 61 m, 5 AUG 1976; ASHFORD, 1 mi. e. Ashford 46°45'25" 121°59'00", 426 m, 14 JUL 1976, 18 AUG 1977; FISH CREEK, Mount Rainier Nat'l. Park 46°47'15" 121°52'55", 914 m, 15 JUL 1976, 17 AUG 1977; MOUNTAIN MEADOWS, Mt. Rainier Nat'l. Park 46°56'46" 121°52'40", 1304 m, 18 AUG 1977; PARADISE, Mt. Rainier Nat'l. Park 46°47'15" 121°43'35", 1585 m, 16 JUL 1976, 16 AUG 1977; NILE CREEK, Snoqualmie Nat'l. Forest 46°52'25" 121°55'25", 1030 m, 13 JUL 1976. Mt. Adams transect. COUNCIL LAKE (Jct. forest rd. 123 & N84), Gifford Pinchot Nat'l. Forest 46°15'35" 121°36'40", 1285 m, 31 JUL 1976.

Oregon: Mt. Hood transect. VERNONIA LAKE, Vernonia 45°51'25" 123°10'40", 163 m, 27 JUL 1976; MUD LAKE, Mt. Hood Nat'l. Forest 45°36'00" 121°47'20", 1085 m, 25 JUL 1976; RED TOP MEADOWS, Mt. Hood Nat'l. Forest 45°17'00" 121°42'25", 1097 m, 4 AUG 1976; MT. HOOD MEADOWS (ski area), Mt. Hood Nat'l. Forest 45°19'45" 121°39'25", 1640 m, 22 JUL 1976.

2.2 Methods

Those parameters which describe the genetic relationships between populations, i.e., levels of migration, interbreeding, and interfertility, can rarely be measured, but must be inferred from observations of character variation. In this study, two sources of variation were used, protein variation as detected by electrophoresis and variation in body colour.

2.2.1 Electrophoretic variation

The basis for the use of electrophoretic techniques to study the degree of genetic relationship between populations has been discussed by Avise (1974) and Lewontin (1974), who noted that much of the protein variation detected by electrophoresis can be interpreted in terms of genetic differences between individuals at single loci and populations can then be characterized in terms of gene frequencies.

Each individual from a sample was analyzed for variants of an esterase and an aldehyde oxidase enzyme. Several other enzymes proved unsuitable (many individuals could not be analyzed, or the genetic basis was unclear).

Reagents, equipment and electrophoretic methods used were those of Rolseth and Gooding (1978) unless indicated otherwise. Polyacrylamide slab gel electrophoresis (6% separating gel, pH 8.2) was used throughout.

Specimens were hand collected, starved for two days,

frozen on dry ice and stored intact at -70°C. Abdomens were pried away from frozen specimens with a scalpel, placed in 0.4 ml of homogenizing buffer, cooled and ultrasonicated at full power for 3 min. using a Braunsonic 500 ultrasonicator. Extract was centrifuged at 10,000 g for 10 min. Aliquots of 0.015 ml of sample were electrophoresed at 50 mA until bromophenol blue marker dye had moved 6.5 cm into the separating gel. Before each esterase run, 0.015 ml of 0.1 M dithiothreitol in homogenizing buffer was added to each aliquot to prevent satellite band formation. Individuals scored in early runs were used as controls in later runs.

Aldehyde oxidase was stained for and gels were washed as described by Rolseth and Gooding (l.c.), except without hypoxanthine in the staining mixture. The esterases were stained with 60 mg Fast Blue RR (Anachemia) in 1 ml of 2% 1-naphthyl acetate (Sigma) and 1.5 ml of 2% 2-naphthyl acetate (Sigma) brought to 25 ml with 0.2 M phosphate buffer, pH 6.0. Staining at room temperature was stopped after 30 min. with 7% acetic acid and gels were then washed in water.

2.2.2 Colour variation

It was hoped that changes in selection pressures with altitude or distance would be mirrored by allozyme frequency differences, but several authors have suggested that characteristics under single gene control may not adequately reflect the spectrum of selective pressures which act on the individual (Berry and Peters, 1976; Soule and Yang, 1973). Berry and Peters (l.c.) suggested that qualitative character variants under polygenic control are more sensitive to differences in selective pressure on different populations. Accordingly, specimens were also analyzed for a complex colour polymorphism.

Adults were assigned to green, copper or melanic colour categories. Some individuals were intermediate in colour form or were of a different colour altogether, and were assigned to an "others" category. The colour of several body features combine to effect the overall colour of the body (Fig. 2); PUNCTURES, which cover the dorsal surface of the body, INTERSPACES between the punctures, and the dorsal surfaces of the LEGS.

The green indiviudals have bright green punctures, with black interspaces, the dorsal surface of the femur and tarsus also being a bright green. A copper body colour is effected two ways: copper (Mt. Rainier) is a form with bright copper punctures, interspaces black, legs entirely copper; in the other form, copper (Mt. Baker), individuals have bright green punctures with shiny bronze interspaces

between punctures, the apex of the femur and tarsus being copper, the remainder of the leg green.

In the melanic form, the basic body colours are distinguishable, but are dull and suffused with black.

2.2.3 Age of adults

Observations of two reproductive characteristics were made to provide an estimate of the number of immature adults in several samples,

a. Sclerotization of the male genitalia. The aedeagus (intromittent organ) does not become heavily sclerotized until well after the beetle has emerged from the pupa (pers. obs.).

b. Presence of eggs in the female. Well-developed eggs are not seen in females which have recently emerged (pers. obs.). The status of females without eggs could not be evaluated, because the observations were made after extraction of enzymes from the abdomens, at which time eggs in some specimens may have been dislodged.

Bauer (1974) reported that adults of Elaphrus riparius L. mature in one to two months, the period from egg to adult being one month. It is possible that adults of E. americanus could mature by the end of their first summer, but there is no evidence to suggest that adults reproduce before their second summer.

2.3 Statistical analyses

Chi-square tests were applied to enzyme data for sample fit to Hardy-Weinberg equilibrium expectations. Comparisons between samples of the number of individuals with particular phenotypes were made by contingency chi-square tests, but in small samples, or where intrapopulation variation was too large, sufficiently large expectations (>5) could not be generated for a valid chi-square test. To obtain some estimate of the relationship of small samples to larger ones, the probability of obtaining the actually observed number of individuals of a certain genotype in a small sample, if that sample had been taken from an observed sample of larger size, was calculated. The probability is given by: $P = C_{(n,r)} p^r (1-p)^{(n-r)}$, (Robbins and Van Ryzin, 1975) where C = binomial coefficient, p = proportion of a genotype in a large sample, r = observed number of individuals of that genotype in the small sample and n = size of the small sample. Where intrapopulation variation was too large to allow a direct comparison of phenotype distributions between samples, the distribution of allele frequencies between samples was compared by Spearman's rank correlation test (Robbins and Van Ryzin, l.c.). Since only two enzymes were examined, quantitative estimates of genetic similarity between populations (Nei, 1972) were not made.

Statistical comparisons of colour variation between samples were not done, because differences between samples, when observed, were of such magnitude that simple inspection

of colour data seemed adequate.

3. Results

3.1 Habitat comparisons between subalpine and lower altitude populations

Populations of Elaphrus americanus above the limit of continuous forest were found in broad subalpine basins (subalpine parkland subzone, Brooke et al., 1970). At Paradise, Mt. Rainier, beetles were collected from early snowmelt sites but only where there was considerable surface drainage of meltwater from adjacent snowpack. These sites were on exposed, gentle slopes which later dried out or became densely vegetated with sedges and herbs, as the season progressed. Beetles were then found downslope on poorly drained gleysolic soils in protected, late-melting snowbed habitats characterized by Carex nigricans sedge communities (Brooke et al., l.c.). Many poorly drained sites along small streams also support this type of sedge community, according to Franklin and Dryness (1973). Samples of E. americanus were collected from such sites also. At lower elevations, snowbed habitats do not occur, and the majority of collections of E. americanus (11 of 20) were made from beaver (Castor canadensis (Kuhl)) dam sites along streams on exposed mud flats where dams had been washed out or had fallen into disrepair.

Subalpine basins seem to provide a suitable habitat over the life span of individual beetles and within a basin,

snowbed habitats are probably stable for thousands of years. Very large populations of E. americanus may be maintained, several hundred individuals were observed in an hour at two different subalpine sites (Bagley Lakes, Paradise). In contrast to subalpine areas, suitable low and mid-elevation sites were widely scattered and relatively small in extent (<30 m^2), and probably because of the limited area of the habitat, lowland sites generally supported small numbers of adults (<50). Habitat instability appears to be a characteristic of low and mid-altitude sites. Five low or mid-altitude sites were revisited after a period of three weeks in July of 1976. At one site, the pool and surrounding mud flats had entirely dried out, a second site was almost entirely dry and three sites were flooded. In contrast to the initial collection at each site, few specimens of E. americanus were found during the second visit. Beetles at these sites had probably dispersed elsewhere as the conditions at each site deteriorated.

3.2 Life cycle comparisons

The life cycle data are most extensive from sites on Mt. Rainier and are probably representative for the Pacific Northwest in general. In 1976, on Mt. Rainier, adults began reproductive activities earlier in the season at mid-elevations than at high elevations, since, in mid-July, reproductively immature males were collected at mid-elevation, but none were seen from a subalpine parkland sample (Table 3). Beetles in the subalpine had probably just become active, as most of the subalpine parkland was still under snow on the day the sample was collected. It is not known whether the mature adults in the subalpine sample had overwintered *in situ* at early snowmelt sites or had flown in from elsewhere. In August, 1977 immature adults predominated in collections taken at all elevations and this probably indicates that reproductive activity started earlier in 1977, the probable reason being that the summer of 1977 was hot and dry, while 1976 was cool and wet.

3.3 Interpretation of enzyme variation

An esterase system designated EST-1 was investigated (Fig. 3), EST-1 bands were distinguished by substrate specificity from bands of two overlapping systems which were expressed only occasionally. The EST-1 bands stained dark brown (hydrolyzed 1- and 2-naphthyl acetate), whereas the second system stained grey (1-naphthyl acetate specific), the third red (2-naphthyl acetate specific). With few exceptions, only single or double band patterns were observed, indicating that EST-1 is a monomer, with each band representing an allele at the EST-1 locus, and double-banded individuals being heterozygous (Hubby and Lewontin, 1966). Twenty-one EST-1 alleles were recognized over 17 samples. Bands, from different individuals, which overlapped in position on gels, were considered to be products of a single allele. However, these "alleles" could be a composite of several alleles whose products have similar electrophoretic mobilities. The alleles were designated alphabetically in ascending order of mobility, EST-1^a; EST-1^b; etc. The mobilities of common allozymes relative to the bromophenol blue front (Rf) are given in Table 1. Hardy-Weinberg expectations for the commonest EST-1 variants were large enough (>5) for statistical treatment only in a few samples. Each of three samples tested conformed to binomial expectations (Table 4).

One area of aldehyde oxidase activity, designated AO-1, was present on gels (Fig. 4). This enzyme appears to be a

dimer, three band phenotypes expressed by the presumed heterozygote (Hubby and Lewontin, l.c.). Ten alleles were recognized and for the commonest alleles, all but one of 12 samples tested conformed to Hardy-Weinberg expectations (Table 5). The sample from Vernonia Lake showed a barely significant excess of homozygotes.

3.4 Patterns of enzyme variation

The data are comparable between years since, of samples collected at three sites in both 1976 and 1977, only one, the collection from Ashford in 1977 differed from the sample taken the previous year (Table 6). Only one other sample (Anderson Creek) did not conform to overall patterns of variation (section 3.4.1), thus no significance is placed on the year to year discrepancy in the Ashford samples.

3.4.1 Low and mid-elevations populations

At low and mid-elevations, EST-1 variation within a population was extremely high (Table 1). Eleven to 15 alleles were recognized per sample (n>30), no allele exceeded a frequency of 0.35 and three to six alleles reached frequencies of >0.10 in a given sample. Frequency of heterozygous individuals ranged from 0.647 to 0.951. Low and mid-elevation samples could not be differentiated from one another by variation at EST-1. Pairwise comparisons between samples of the distribution of EST-1 phenotypes were not attempted because the observed number of individuals of any

given phenotype was too small to generate sufficiently large expectations (>5) for a chi-square test. These samples were compared by a rank correlation of allele frequencies (Table 6) which indicates that low and mid-elevation samples have similar EST-1 allele frequencies, except Ashford (1977) and Anderson Creek.

The AO-1 intrapopulation variation at low and mid-elevations was not as great as EST-1 diversity. Four to nine alleles were observed per sample and at all sites AO-1^e and AO-1^g predominated, with AO-1^i consistently third most frequent; AO-1^e frequency 0.380 to 0.556, AO-1^g frequency 0.227 to 0.525. Proportion of heterozygous individuals ranged from 0.420 to 0.636 (Table 2).

Pairwise comparisons between samples at low and mid-elevations for three AO-1 phenotypes, e/e, e/g, g/g (see Table 5 for number of individuals) indicated that two samples-Anderson Creek and Ashford (1977) were significantly different from most other samples. Ashford (1977) was also significantly different from the sample collected at the same site the previous year (chi-square, 3 df = 9.744, $p<.025$). However, Ashford (1976) was not different from any other samples except Anderson Creek and since the same AO-1 alleles predominate in all low and mid-elevation samples (Table 6), no significance is attributed to observed differences.

3.4.2 Subalpine parkland samples

Intrapopulation variation of EST-1 in most subalpine parkland samples was less than in lower altitude samples (Fig. 5, Table 1). Fewer alleles were seen (six to nine) and a smaller number of alleles (two to four) exceeded a frequency of 0.10 in a given sample. Commoner alleles accounted for a larger proportion of the total number of EST-1 genes in a sample, the proportion (given as a mean) accounted for by the three commonest alleles being 0.82 (range 0.769 to 0.835) of the total in the larger subalpine samples (n>30) and 0.54 (0.462 to 0.646) in the low and mid-elevation samples. However, no EST-1 alleles exceeded a frequency of 0.666 thus the proportion of heterozygous individuals was still high (0.653 to 0.729 in larger samples).

The variation between subalpine parkland sites was marked, that is, the EST-1 allele frequencies on Mt. Baker (Bagley Lakes) and Mt. Rainier (Paradise) were very different, the commonest EST-1 alleles at Paradise were j, i, l; at Bagely Lakes m, i, f. The small Tusk Mtn. subalpine sample (Mimulus Lake, n = 15) also appeared to have distinctive EST-1 allele frequencies, alleles i and m being most common. Pairwise comparisons between subalpine sites on Mt. Rainier and Mt. Baker, of the four commonest phenotypes; i/j, j/j, i/m, m/m (see Table 4), indicated that EST-1 differences were statistically significant (chi-square, 4 df = 33.884, $p<.001$). The Tusk Mtn. sample was also

significantly different from the Mt. Rainier and Mt. Baker samples (phenotypes compared: i/i, i/m), however, calculated expectations were too low for a valid test. Thus, the probability of drawing the Tusk Mtn. sample from these larger samples (with respect to i/i and i/m) was calculated (Table 7). These calculations also indicate that Tusk Mtn. is distinct in frequencies of EST-1 alleles.

Intrapopulation variation of AO-1 (number of alleles, number of common alleles) was reduced in most subalpine parkland samples (Fig. 6, Table 5). The allele AO-1^{e} predominated on Mt. Baker and Mt. Rainier (frequency range: 0.863 to 0.913) and heterozygosity was strongly reduced (0.152 to 0.244). This allele predominated in the Tusk Mtn. sample also. Mt. Rainier and Mt. Baker subalpine samples had a similar number of individuals with the AO-1 phenotypes c/e and e/e (chi-square was not significant in a contingency table test). The small sample from Tusk Mtn. was also not distinct from larger subalpine samples (Table 7).

In contrast to the three more northern mountains in this study, Mt. Hood does not appear to have a distinct subalpine population. In the small subalpine sample (Mt. Hood Meadows, n = 18) no EST-1 allele exceeded a frequency of 0.222 and eight alleles were seen (Fig. 5, Table 1). Thus, the pattern of EST-1 variation resembles that at lower altitudes. AO-1 variation was also not reduced (Fig. 6, Table 2). The alleles AO-1^{e} and AO-1^{g} were present at nearly equal frequencies and the proportion of heterozygous

individuals was 0.533. Calculation of the probability of drawing the small Mt. Hood Meadows sample from lower altitude samples with respect to AO-1 phenotypes; e/e, e/g and g/g, also indicated relationship of this sample with lower altitude samples (Table 7). Allele frequencies of AO-1 and EST-1 at Mt. Hood Meadows were positively correlated with allele frequencies in lower altitude samples (Red Top Meadows) but the correlations were not statistically significant (Table 6).

3.4.3 Subalpine forest samples

Two samples were taken from the forested lower reaches of the subalpine zone (subalpine forest subzone, Brooke et al., 1970). The Council Lake sample on Mt. Adams was not different in frequency of either AO-1 or EST-1 alleles from low and mid-elevation samples (Table 1, 2 and 6). The small (n=12) Mountain Meadows sample on Mt. Rainier appeared to fit the variation pattern of EST-1 at lower altitudes (Fig. 5), but this could not be tested statistically. In this sample AO-1^e predominated as at higher altitudes (Fig. 6) and the probability is high that with respect to AO-1^e, this sample was drawn from a subalpine parkland sample (Table 7).

3.4.4 Patterns of enzyme variation-summary

The variation in enzymes AO-1 and EST-1 indicated that there are three distinct subalpine parkland populations: Tusk Mtn., Mt. Baker, and Mt. Rainier. Transition from the widespread low altitude population to the subalpine population occurred over a maximum altitudinal distance of 600 m on Mt. Rainier. The sample from Mountain Meadows on Mt. Rainier indicates that the variation patterns of EST-1 and AO-1 are not congruent in the transition zone and that altitudinal effects on AO-1 occur first. No distinct subalpine population was found on Mt. Hood, and as for the Mt. Adams sample from the subalpine forest subzone (Council Lake), it does not show altitudinal effects, thus, there may not be a distinct subalpine population on Mt. Adams either.

3.5 Patterns of colour variation

Colour variation appears to be independent of enzyme variation since the distribution of EST-1 and AO-1 alleles within samples are not correlated with colour morph (Table 8). This colour variation data (Table 9) supports much of the interpretation of enzyme data, e.g., Subalpine parkland samples on Tusk Mtn. (Mimulus Lake), Mt. Baker (Bagley Lakes) and Mt. Rainier (Paradise) all had a high frequency of copper coloured individuals while lower altitude samples did not. Distinctiveness of certain subalpine populations was partly verified since the copper colour morph found on Mt. Rainier was not found on Mt. Baker or Tusk Mtn. The

altitudinal transition for colour variation may be as abrupt as with the esterase enzyme, since no copper individuals were observed from the subalpine forest site on Mt. Rainier (Mountain Meadows).

Melanic individuals are common only on Mt. Hood at mid and high elevations (Mud Lake, Red Top Meadows, Mt. Hood Meadows) and on Mt. Adams (Council Lake). This is inconsistent with enzyme variation data which did not indicate differentiation of any low or mid-altitude samples.

Presence of melanics in the subalpine Mt. Hood sample (Mt. Hood Meadows) indicated, as did the enzyme data, again that there is no distinctive subalpine population on Mt. Hood.

4. Discussion

4.1 Genetic interpretation of enzyme and colour polymorphism data

Electrophoretic mobility may be influenced by non-genetic factors (Ressler, 1973). No attempts were made to cross E. americanus polymorphs in the laboratory and thereby establish more directly, that the enzyme variation has a genetic basis, but four lines of indirect evidence support such an inference:

(1) Electrophoretic banding patterns. The observed band patterns were consistent with control by multiple alleles at single loci. Each individual expressed only one of two patterns for each enzyme: single band (homozygote); two bands (heterozygote for EST-1); three bands (heterozygote for AO-1). Similar patterns of esterase bands have been observed in other insects and shown by genetic experiment to be allelic variants at single loci (Est-6 in Drosophila melanogaster, Wright (1963); EST-6 in Aedes aegypti, Saul et al. (1976)). Similar aldehyde oxidase band patterns have been reported and shown to be allelic variants of a dimeric enzyme for Drosophila melanogaster (Dickinson, 1970); and for Glossina morsitans morsitans (Rolseth and Gooding, 1978).

(2) Stability of allele frequencies. Samples taken from the

same sites in 1976 and 1977 on Mt. Rainier did not differ substantially in allele frequencies (Table 6) despite: (a) being one generation apart; (b) within generation age differences, the 1976 samples consisting mainly of over-wintered reproductively mature individuals, the 1977 samples composed of immature adults (Table 3); (c) climatic contrasts in 1976 and 1977.

(3) Patterns of variation. The same electrophoretic variants were present in low altitude and subalpine populations, only their frequencies were different.

(4) Hardy-Weinberg equilibrium. Almost all samples tested conformed to binomial expectations for AO-1 and EST-1 phenotypes (section 3.3).

Taken as a whole, the data are consistent with the hypothesis that observed electrophoretic variation of each enzyme reflects genetic variation at a single locus.

There is no evidence of genetic control of colour variation in Elaphrus species, but Young (1965) suggested, on the basis of various crosses, that several co-dominant genes control extent of bronze colouration on the elytra of a hydrophilid water beetle Tropisternus collaris F. Genetic control of the colour polymorphism shown in E. americanus is assumed and since several characteristics contribute to overall body colour, a number of genes probably control the polymorphism, with different genes responsible for expression of the copper (Rainier) and copper (Baker)

morphs, since they are so different from one another (section 2.2.2).

4.2 Origin of subalpine populations: Possibilities

The general question as to how Elaphrus americanus extended its altitudinal range could be rephrased, "How can the presence, on various mountains, of two strongly differentiated populations which replace each other abruptly with altitude, be accounted for?" Huxley (1942) discussed two possibilities. If selection pressures differ markedly in each of two habitats (altitudinally delimited in this study), a continuous population might become differentiated into two subpopulations, each adapted to one of the habitats. The contact zone between the populations would be one of primary intergradation (Mayr, 1963). Alternatively, there may be secondary contact between the two populations which differentiated from one another in geographical isolation. It is argued that the contact zone between subalpine and low altitude forms is a primary one.

4.3 Primary intergradation: evidence

Several lines of evidence indicate the contact zone is a primary one: an abrupt change in environmental gradients with altitude; the likelihood of strong differential selection pressures in the subalpine zone; the possibility of restricted gene flow between low and high altitude populations through a temporal separation of reproductive periods.

4.3.1 Environmental gradients

Environmental conditions differ dramatically over a short elevational distance in the study area. Brooke et al., (1970) reported that on the seaward slopes of the Coast Range in British Columbia (Tusk Mtn. area of this study), marked accumulation of winter snowfall occurs only above the lower limit of winter freezing temperatures at 990 m., which marks the lower boundary of the subalpine forest subzone (Brooke et al., l.c.) and corresponds precisely to the transition area between subalpine parkland and mid-altitude populations of E. americanus on Mt. Rainier. Below the zone of snow accumulation, the activity of E. americanus populations starts considerably earlier in spring (records seen for late May), while the earliest collections from subalpine elevations are dated from mid-July.

4.3.2 Role of selection

Genetic differentiation of continuous populations across sharp environmental gradients has been demonstrated for a variety of sessile organisms such as grasses on metal contaminated soils (McNeilly and Bradshaw, 1968) and mussels in relation to water salinity (Koehn et al., 1976). Fewer examples are known for vagile organisms. A population of Drosophila melanogaster in a wine cellar had a higher tolerance to alcohol than the population in an adjacent vineyard and this tolerance was maintained despite considerable gene flow into the cellar (McKenzie, 1975). Platt and Brower (1968) reported an abrupt reversal of selective forces accounted for a switch-over from mimetic to disruptive colour forms in the Limenitis arthemis-astyanax butterfly complex, the astyanax form found only within the range of its model, Battus philenor L. In a subdivided population of sulfur butterfly, Colias meadii Edw. in Colorado, Johnson (1976) demonstrated significant differences in isozyme frequencies between montane meadow and subalpine populations, in spite of dispersal of 10% of the individuals between adjacent subpopulations. Such studies support the contention that strong selection pressures may lead to differentiation within populations in spite of gene flow (Ehrlich and Raven, 1969).

Considerable differences in selective pressures probably occur between subalpine and lower altitude sites. Ferenz (1975) studied physiological differences between

continental (Central European) and subarctic (Swedish Lapland) populations of the ground beetle species Pterostichus nigrita F. Subarctic P. nigrita larvae showed reduced mortality rates at low temperatures, developed faster at high temperatures and achieved greater weights, while adults were able to initiate and complete gonad maturation under long day photoperiod, individuals from central Europe requiring short day exposure. These differences were interpreted as adaptations of northern populations to a short summer activity period under long day conditions, interrupted by frequent cold spells. Similar adjustments could be expected to occur in subalpine populations of Elaphrus americanus which must cope with short summers, cold spells and are active only during long day conditions.

Can observed patterns of enzyme and colour variation be attributed to selection? Other workers have studied geographic patterns of intraspecific variation of highly variable enzyme systems and have argued that selection maintained the variation (e.g. Burns and Johnson, 1971) but conclusions based on studies of geographic patterns alone have been questioned (Lewontin, 1974). In this study, predominance of the allele AO-1^e in several otherwise differentiated subalpine populations does suggest selection for this allele at high altitude, but as in most studies of this kind, it is not known what selective advantage the AO-1 allozyme might have, physiological or otherwise. Factors

which affect frequencies of AO-1 alleles do not precisely correspond to those influencing EST-1 variation, since the sample from the transition zone on Mt. Rainier fits the subalpine populations in AO-1 variation but not in EST-1 allele frequencies. In an analogous situation, Johnson (l.c.) observed several different clinal patterns of isozyme variation in a population of Colias meadii along an elevational transect across the subalpine zone on a Colorado mountain and he attributed the patterns to selection.

The copper coloured individuals of E. americanus are probably at an advantage at high altitudes since the copper forms are common only in the subalpine parkland and, most likely, have been derived independently on Mt. Rainier and Mt. Baker (derivation of the copper (Rainier) morph from the copper (Baker) form requires two steps - bronze to black interspaces, green to copper punctures, but only one step is required from the green morph, green to copper punctures). Protection from predation and physiological advantages have both been implicated in maintenance of other colour polymorphisms in insects (Kettlewell, 1961), but correlation between background colour and body colour was not observed in the field for E. americanus populations.

Patterns of colour and isozyme variation between samples were largely congruent with one exception. The mid-altitude populations in southern Washington and northern Oregon could be distinguished from other mid-elevation populations in the high number of melanic individuals but

did not differ in frequencies of enzyme variants. A comparable discrepancy between morphological and isozyme data was reported for island populations of spittle bug Philaenus spumarius L. by Saura et al., (1973). Different selection pressures were thought to act on enzyme and colour polymorphisms, since different colour variants predominated on each island, while the same enzyme variants were common on all islands.

Environmental factors may retard differentiation of subalpine forms on some mountains. The Pacific Northwest experiences summer drought and by late summer in subalpine areas, most individuals of E. americanus are found near permanent ponds and small streams. Permanent ponds are rare in subalpine areas on Mt. Hood and Mt. Adams and in some years conditions may be too dry in these areas to maintain populations of E. americanus. The one sample from a subalpine area on Mt. Hood was from a man-made site, a drainage ditch in a ski area.

4.3.3 Role of gene flow

In addition to selection, levels of migration between adjacent populations also affect degree of differentiation of those populations. For example, selective advantage of alcohol tolerant individuals of Drosophila melanogaster was not high enough, in relation to the observed amount of gene flow into the cellar, to maintain a distinct, alcohol-tolerant, wine cellar population (McKenzie, 1975).

He demonstrated that migration into the cellar is restricted to one period of the year and postulated that only alcohol tolerant dispersers make the move successfully.

What are present levels of gene flow between subalpine and mid-elevation populations of E. americanus? If considerable movement of adults occurred during the study, significant deviations from Hardy-Weinberg expectations should have been observed (see Koehn et al., 1976), but in this study, subalpine and mid-altitude samples conformed to Hardy-Weinberg expectations. Excesses of heterozygotes were observed as commonly as heterozygote deficiencies.

If dispersal rates are low between subalpine and mid-elevation populations, then only a few migrants would be present at any time at a site, but this dispersal could not be detected by examining samples for deviation from binomial expectations since the test for Hardy-Weinberg equilibrium is not sensitive enough (Wallace, 1968). Since populations were only partially differentiated at each of the loci studied, the problem was compounded.

Since the effects of dispersal could not be detected at the population level, it was hoped that individuals could be identified as to origin. Coluzzi and Bullini (1971) used electrophoretic variants as markers to identify hybrid individuals in a study of gene flow in mosquitoes. In E. americanus, one common electrophoretic variant was almost restricted to a subalpine population - EST-1 which was present at frequencies of 0.25 to 0.35 in the subalpine

population on Mt. Rainier (Paradise). One individual from the transition zone sample (Mountain Meadows) was heterozygous for EST-1^{J} and one EST-1^{J} heterozygote was collected still lower down at Fish Creek in 1977. It was not possible to determine whether these EST-1^{J} genes originated by introgression from the high altitude population, or if EST-1^{J} is generally present at low frequency (<2%) at lower altitudes, because differences in allele frequencies were not of sufficient magnitude to allow identification of individuals as to their origin. Ayala and Powell (1972) were able to use single loci to differentiate individuals of two sibling species, since different alleles were nearly fixed at one or more loci, but a large number of partially differentiated loci would have to be used to identify individuals of E. americanus as to origin.

Two observations suggest that dispersal into and out of subalpine areas may be restricted:

(1) The delayed availability of subalpine habitats due to persistence of snow probably restricts movement of individuals from lower altitudes into the subalpine zone and vice versa until late in the season and by this time some portion of reproductives at lower altitudes will have been replaced by immature adults, and these individuals could not affect the genetic makeup of subalpine populations until the next summer.

(2) Since subalpine habitats are stable over many years, while the sites at lower altitudes may not persist over a

single season, individuals from subalpine sites may not disperse to lower elevations to the same extent as beetles from low and mid-altitudes move higher. Gilbert and Singer (1973) reported that adults of the butterfly Euphydryas editha (Boisduval) from an unstable habitat dispersed more readily than adults from a population in a stable habitat and this difference probably had a genetic basis.

With restricted levels of dispersal of individuals between subalpine and mid-altitude populations, selection could be responsible for the observed differentiation.

4.4 Secondary intergradation

Geographic isolation and differentiation of various populations during the Pleistocene, one or more of which became subalpine forms, seems unlikely. Mountain ranges in the study area, from Canada to southern Washington, were largely covered by ice during glacial stages of the Pleistocene (Denny, 1970) at which times montane populations of E. americanus were presumably destroyed or displaced to lower elevations south of the ice sheets (Fig. 1). Tundra communities probably occurred along the ice margins (Heusser, 1972), and could have supported a string of tundra-adapted E. americanus populations, but since forests were probably always in close proximity to the ice margin (Heusser, l.c.), it would still be necessary to account for origin of lowland tundra-adapted populations of E. americanus from lowland forest populations nearby. If one or

more tundra-adapted populations did exist south of the ice sheets, then with the retreat of glaciers at the end of the Pleistocene, tundra-adapted populations moved with the glaciers to higher elevations, adapted to higher altitudes, and diverged from one another as populations became confined to isolated subalpine areas.

Establishment of subalpine populations might also be accounted for by a "founder event" (Mayr, 1963; Carson, 1965), during which, initial colonization was achieved by only a few individuals, who carried only a small portion of the genetic variation of the parent population. Rapid genetic adjustments and differentiation occurred as the population expanded in the new habitat.

The strongest argument against the founder hypothesis is the following evidence for multiple dispersals into the subalpine areas:

(1) Adults fly well and potential physical barriers such as the Columbia River basin or the Cascade Crest do not appear to influence patterns of variation in E. americanus.

(2) Beetles were collected at all suitable sites within the transition zone, despite the fact that habitats are rare at those elevations because slopes are generally steep. This indicates that the transition zone is not an effective dispersal barrier.

(3) Females of several Elaphrus species lay eggs in habitats not characteristic of those species when, according to Goulet (1978), various riparian habitats are used as rest

stops during dispersal flights. The females from lower elevations may often "test out" new habitats in this way when dispersing.

(4) A large proportion of alleles seen in lower altitude samples are present in samples from subalpine populations.

(5) Samples from subalpine areas on some mountains (Mt. Hood, possibly Mt. Adams) do not differ from lower altitude samples.

The arguments in points (4) and (5) are complicated by observations that enzyme variants with identical mobilities often show heterogeneity in various properties such as resistance to denaturation by heat or urea. This indicates that several alleles at a locus may code for enzymes with identical electrophoretic mobilities and allows the possibility that such electrophoretic variants may arise independently in different populations (Reviewed by Selander, 1976; Throckmorton, 1977).

The alternative historical synopsis, in terms of a habitat extension in post-Pleistocene times, is as follows: The retreat of Cordillerean glaciers at the end of the Pleistocene allowed recolonization of subalpine areas through multiple dispersal of individuals from populations of what are now low and mid-elevation forms. Successful establishment in the subalpine zone probably occurred along the edges of ponds, which are usually in close proximity to snowbanks that persist for most of the summer. Adaptation of subalpine populations to local conditions was necessary and

was also enhanced during cool, wet periods when heavy snowfalls would result in short seasons and some allochronic separation from lower altitude populations.

4.5 Reproductive relationships

In the absence of evidence for gene flow between subalpine and mid-elevation populations, it must be asked whether these populations are reproductively isolated from one another. The genetic relationships between subalpine populations are also unclear since they are isolated from one another by distance.

4.5.1 Populations on a mountain

The overall degree of differentiation between populations might be used as an indicator of reproductive isolation, but the observed isozyme differentiation between subalpine and mid-altitude populations is not conclusive since: (1) similar levels of differentiation may be observed both within and between species of *Drosophila* (Throckmorton, 1977; Zouros, 1973); (2) the sample from the transition zone on Mt. Rainier (Mountain Meadows) shows both mid-altitude and subalpine influences.

The strongest evidence for speciation is the ability of subalpine individuals to use late snow-melt sites towards the end of the first summer and after overwintering, then resume activity in early snow-melt habitats in the second summer. This is a considerable behavioural specialization

since E. americanus is not associated with snowmelt at lower altitudes. If in fact the mid-elevation and subalpine populations are to some degree reproductively isolated from one another by behavioural specialization, the initial differentiation could still have arisen by disruptive selection across a sharp environmental gradient (see Maynard Smith, 1966).

4.5.2 Subalpine populations on different mountains

Gene flow between subalpine populations on Mt. Rainier and Mt. Baker may occur since the allele EST-1 was found at a frequency of 0.30 in samples from the subalpine parkland of Mt. Rainier and at a lower frequency (0.07) on Mt. Baker. Clinal variation of EST-1 through intervening populations would then be expected, but samples with which to test this possiblity are not available. If, as has been suggested above, subalpine individuals do not disperse extensively, EST-1^{J} may have been incorporated independently into the two subalpine populations from the lower altitude populations on each mountain, or there are actually two alleles, with each allozyme having the same electrophoretic mobility by convergence through independent mutations. In all these situations, slight selective differences on each mountain could account for the present difference in frequency of EST-1^{J}, although this would be difficult to demonstrate (Lewontin, 1974).

4.6 General considerations

4.6.1 Prevalence of microgeographic races

Genetic adaptations of local populations of animals to the immediate environment are generally physiological and such adaptations are often not reflected in readily discerned phenotypic differentiation. In contrast, locally adapted populations of plants can often be recognized easily, since conspicuous morphological modifications are generally involved (Mayr, 1963), so that much of the work on microgeographic race formation has been done on plants. In animals, protein variation has provided a rich source of polymorphisms with which to study population differentiation and has revealed in this study and in works cited above that rather marked differentiation may occur over short distances in populations of even very vagile animals. Specifically, with respect to the present study, wide-ranging species of insects may commonly have populations adapted to high-altitude conditions on the mountains of western North America. Freitag (1965) studied several colour polymorphisms of various subalpine populations of the tiger beetle species, Cicindela depressula Casey, from the same mountains as in this study. Each population had distinct frequencies of colour morphs, but from his data, it is not possible to determine if relationships between low and high altitude populations (recognized as separate subspecies) are similar

to those observed in Elaphrus americanus. Other examples of altitudinal differentiation of vagile insects include Italian populations of Maniola jurtina L. and various European populations belonging to the Pieris napi-bryoniae complex (reviewed in Ford, 1975).

4.6.2 Microgeographic races: evolutionary potential

Evolutionary potential of populations in peripheral areas, whether on mountains or elsewhere, may depend on local circumstances. In this study, where environmental conditions were too severe (Mt. Hood), populations in subalpine areas appeared to be maintained by immigration of individuals from lower altitudes, but where conditions were more favourable, (populations were protected from the effects of drought), distinctive subalpine populations were found. Greenslade (1968) suggested that ground beetle populations in the severe conditions at the highest altitudes on Scottish mountains, maintain their numbers solely by immigration from lower elevations. Two sorts of data put the long-term evolutionary impact of microgeographic races in doubt: (1) Quaternary beetle fossils (Coope, 1970); (2) The flora of metal-contaminated soils in areas of great geological age (Wild and Bradshaw, 1977).

There is little fossil evidence for any morphological evolution of beetle species in the upper half of the Quaternary (Coope, l.c.) and Goulet (1978) assigned six million year old Elaphrus fossils to extant species. Coope

noted the contrast of this morphological conservatism with the existence of races of ground beetles which are most certainly of post-Pleistocene origin and he suggested that formation of local races of beetle species is simply a function of the broad adaptability of widespread species and may not be reflected in long-term evolutionary changes.

The widespread occurrence of many plant species with populations adapted to localized areas of soil contaminated by heavy metals led Wild and Bradshaw (l.c.) to predict that there should be a high level of endemism in metal contaminated areas of great geologic age. This was observed only in areas of large extent and the authors concluded that most instances of local coadaptation simply involve too few individuals in too small an area to allow the local population to survive over the long term.

Populations of Elaphrus americanus may readily adapt to local conditions which exist at present in the subalpine regions of the Pacific Northwest, but the probability of extinction of these populations over short periods of time may be high due to the instability of the area, geological (vulcanism) and climatic (alpine glaciation). Nonetheless, these populations may contribute to the overall adaptability of the species through the introgression into lower altitude populations of "subalpine" genes (possibly EST-1[3]). In the grass Agrostis tenuis Sibth., workers have studied the potential for the evolution of metal-tolerant populations from non-tolerant populations and found that the greatest

initial response to selection was from non-tolerant populations which were growing adjacent to tolerant populations and had acquired a considerable reservoir of metal tolerance genes from the the tolerant populations through gene flow (reviewed in Antonovics et al., 1971). Zoogeographers have long recognized that mountain faunas often show high levels of endemism and are to a large extent derived from the surrounding low altitude fauna. Darlington (1971) attributed such patterns to the stability of montane habitats, particularly in the tropics, where few, if any, species of ground beetles occupy a wide altitudinal range, but in contrast, Greenslade (l.c.) noted that many species of ground beetles occupy a wide altitudinal range on Scottish mountains. Thus, the evolutionary influences on the biota of mountain areas at high latitudes may simply not be comparable to those in mountainous tropical areas.

4.6.3 Microgeographic races: taxonomic implications

How are microgeographic races, such as subalpine populations of E. americanus, to be treated taxonomically? Mayr (1963) pointed out the controversy in the botanical literature regarding this topic. To the present, in most groups, zoologists have not been faced with the problem because of the difficulty in recognizing locally differentiated populations, but with the increased use of protein polymorphisms, pressure may increase to recognize

microgeographic races formally. A difference of opinion about such recognition will continue because of a dual function of "species" and "subspecies" on one hand as taxonomic units and on the other as evolutionary concepts. Mayr (l.c.) and others (Ross, 1974) suggest that formal recognition of differentiated populations, by naming of subspecies, should be restricted to distinctive forms, usually recognizable by external structural features, which have virtually completed the speciation process. Since there is doubt about the relationships between high and low altitude populations of E. americanus, subalpine populations should not be formally recognized as subspecies.

4.7 Concluding remarks

The relationship between various populations of E. americanus from the Pacific Northwest remains unsolved. Much of the analytical power of using discrete polymorphisms, electrophoretic or otherwise, is lost, because the populations in question do not overlap in space, so that the level of dispersal between these populations becomes important. The interpretation of the observed geographic patterns of differentiation hinges, in large part, upon resolution of two controverial areas in biology, the adaptiveness of protein variation, and the relative importance of gene flow versus selection to evolutionary processes. If the enzyme polymorphisms observed in these populations are of no adaptive value, then the lower

altitude and subalpine populations must be considered to be reproductively isolated from one another, since it is established that a small amount of gene exchange between populations will prevent differentiation of those populations, all things being equal (Lewontin, 1974). If these polymorphisms are adaptive, then the possiblility of distruptive selection in the face of gene flow, must be considered. One way of demonstrating gene flow was suggested, that of identifying hybrid or backcross individuals, this being dependent upon discovery of enzyme loci with alternate allozymes virtually fixed in different populations, or on the use of a large number of partially differentiated enzymes. Use of mark-release-recapture methods could be a more direct way to demonstrate dispersal, but it might be almost impossible to locate dispersers, owing to the large extent of the area under study. However, the suggestion made here, that the frequency of dispersal differs for low altitude and subalpine populations, could be tested, and also the possibility that reproductively mature individuals from lower elevations, may cease to disperse before subalpine areas become free of snow. A drawback to these methods would be that only dispersal, and not gene flow, would be demonstrated. Releasing large numbers of individuals from low altitude populations into a high altitude population and vice versa, tracing their effect on the recipient population by the use of genetic markers, would be a more direct approach, but the techniques for such

a manipulation are not available for ground beetles. Another approach could be to collect a series of large samples from transitional populations spanning the distance from Mt. Rainier to Mt. Baker. If there is gene flow, clinal variation of enzymes (EST-1) between subalpine populations should be reflected by changes in relative abundance of alleles in the corresponding transition zone populations, but if the transition zone populations are independent of high altitiude populations, then clines through the subalpine populations should not affect patterns of variation in the corresponding transition zone samples. If indeed, the interpretations made here are correct, other organisms should show parallel changes across the forest - subalpine parkland transition zone. Most of the other species in the genus Elaphrus have a wide geographic range and are specialized to one sort of temporary riparian habitat or another, and abrupt changes in the genetic makeup of populations, corresponding to steep environmental gradients, may be found, perhaps in E. californicus Man., populations of which occur in adjacent forest and prairie environments.

The genus Elaphrus is quite successful in terms of the number of individuals and the wide distribution of various species, but relatively depapurate in number of species. It is a matter of speculation as to why other carabid genera have many more species. The level of polymorphism is very high in Elaphrus species (per. obs.), and it is possible

that high levels of enzyme polymorphism are inversely correlated with the frequency of speciation events. These high levels of polymorphism might also be correlated with specialization to a temporary, unpredictable habitat, but these correlations have been attempted for other organisms, with inconclusive results. Another approach to this problem could be to look at the variance in quantitative characteristics within and between populations of ground beetle species in various genera, the prediction being that much of the variation in Elaphrus spp. is containied within populations, variation in more speciose groups distributed between populations. It is possible that the niche which Elaphrus species occupy, is sufficiently restrictive and specialized, that these beetles rarely have an opportunity to speciate and adapt to new ways of life.

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Table 1. Classification of variation of EST-1 in samples of Elaphrus americanus from the Pacific Northwest. Number of alleles common to samples taken in 1966 and 1977 from the same site are given in brackets. Relative mobility (Rf) is given with respect to position of bromophenol blue dye.

Locality	Sample size	Est-1 allele frequencies									Number of alleles	Proportion heterozygous individuals
		f	h	i	J	k	l	m	q	others		
Mimulus Lake****	15	-	-	.666	-	-	-	.300	.033	-	3	.466
Anderson Creek	44	.045	.113	.261	-	.022	.102	.238	.147	.072	11	.840
Bagley Lakes****	48	.093	.031	.135	.072	.010	.010	.541	.083	.041	9	.729
Brooklyn Road	42	.071	.095	.190	-	.166	.083	.154	.142	.099	13	.833
Ashford 1976	51	.098	.098	.166	-	.225	.088	.088	.186	.051	11	.647
Ashford 1977	40	.100	.150	.075	-	.062	.125	.187	.087	.214	15 (11)	.825
Fish Creek 1976	50	.020	.200	.120	-	.140	.060	.160	.120	.180	17	.780
Fish Creek 1977	49	.010	.142	.122	.010	.112	.153	.183	.112	.156	12 (12)	.734
Mountain Meadows**	12	.125	.125	.041	.041	.166	-	.375	.125	-	7	.916
Paradise 1976****	49	.030	.061	.306	.244	.010	.285	.010	.030	.020	9	.653
Paradise 1977****	50	-	.130	.280	.390	-	.150	.010	.040	-	6 (6)	.700
Nile Creek	50	.010	.130	.030	-	.180	.060	.170	.220	.180	13	.700
Council Lake**	37	.054	.148	.054	-	.243	.108	.148	.175	.070	10	.675
Vernonia Lake	50	.030	.200	.060	-	.110	.050	.190	.170	.190	13	.760
Red Top Meadows	49	.030	.153	.061	-	.132	.163	.204	.081	.206	14	.755
Mt. Hood Meadows****	18	.027	.222	.111	-	.194	.027	.222	.111	.083	8	.777
EST-1 Rf values		.299	.342	.364	.385	.400	.414	.457	.514			

**subalpine forest ****subalpine parkland - allele not observed.

Table 2. Classification of variation of AO-1 in samples of Elaphrus americanus from the Pacific Northwest. Number of alleles common to samples taken in 1976 and 1977 from the same site are given in brackets. Relative mobility (Rf) is given with respect to position of bromophenol blue dye.

Locality	Sample size	AO-1 allele frequencies						Number of alleles	Proportion heterozygous individuals
		c	d	e	g	i	other		
Mimulus Lake****	15	.066	.066	.866	-	-	-	3	.266
Anderson Creek	44	.034	-	.556	.227	.181	-	4	.636
Bagley Lakes****	46	.043	-	.913	.032	.010	-	4	.152
Brooklyn Road	43	.023	.034	.500	.279	.139	.046	7	.627
Ashford 1976	50	-	.040	.500	.380	.080	.010	5	.480
Ashford 1977	40	.062	.025	.262	.525	.100	.025	6 (5)	.550
Fish Creek 1976	51	-	.029	.392	.411	.127	.041	6	.607
Fish Creek 1977	51	.019	.068	.450	.333	.127	.019	6 (6)	.568
Mountain Meadows**	12	-	.041	.708	.125	.125	-	4	.250
Paradise 1976****	50	.160	.010	.780	.050	-	-	4	.240
Paradise 1977****	49	.163	-	.836	-	-	-	2 (2)	.244
Nile Creek	50	-	.010	.470	.400	.100	.002	5	.660
Council Lake**	38	.026	.026	.381	.447	.065	.052	8	.631
Vernonia Lake	49	.020	.030	.500	.306	.081	.050	9	.489
Mud Lake	50	.030	-	.540	.240	.180	.010	5	.500
Red Top Meadows	50	-	.030	.430	.460	.060	.020	6	.560
Mt. Hood Meadows****	15	.066	-	.433	.466	.033	.002	5	.600
Rf values		.369	.373	.380	.391	.401			

** subalpine forest ****subalpine parkland - allele not observed.

Table 3. Variation in occurrence of eggs in females and in sclerotization of the aedeagus of males for samples of Elaphrus americanus collected on Mt. Rainier, Washington in July, 1976; August, 1977. Number of individuals observed in each class is given in brackets.

Locality	Elevation	Females: proportion without eggs		Males: proportion with aedeagus not sclerotized	
		1976	1977	1976	1977
Ashford	426 m	0.577 (26)	1.000 (25)	0.167 (24)	1.000 (14)
Fish Creek	914 m	no data	1.000 (29)	no data	0.778 (18)
Paradise	1585 m	0.231 (26)	1.000 (26)	0.000 (24)	1.000 (22)

Table 4. Comparison of observed and expected (brackets) number of individuals under assumption of Hardy-Weinberg equilibrium for commonest EST-1 phenotypes in samples of Elaphrus americanus from the Pacific Northwest.

Locality	Sample size	EST-1 phenotypes							chi-square	df
		i/i	i/j	i/l	j/j	j/l	l/l	other		
Paradise 1976	49	7 (4.6)	5 (7.3)	6 (8.5)	4 (2.9)	7 (6.8)	6 (6.0)	14 (15.0)	4.300	3
Paradise 1977	50	4 (3.9)	10 (11.0)	4 (4.2)	9 (7.6)	4 (5.8)	2 (1.1)	17 (16.5)	1.651	3
		i/i	i/m	m/m	other					
Bagley Lakes	43	1 (0.9)	10 (7.0)	10 (14.0)	27 (26.1)				2.477	1

Table 5. Comparison of observed and expected (brackets) number of individuals under assumption of Hardy-Weinberg equilibrium for commonest AO-1 alleles in samples of Elephtus americanus from the Pacific Northwest.

Locality	AO-1 phenotypes e/e	e/g	g/g	other	chi square 1 df
Anderson Creek	14 (13.5)	12 (11.1)	1 (2.2)	17 (17.1)	0.774
Brooklyn Road	11 (10.8)	12 (12.0)	3 (3.3)	17 (17.0)	0.033
Ashford 1976	14 (12.5)	14 (19.0)	11 (7.2)	11 (11.3)	3.507
Ashford 1977	3 (2.7)	7 (11.0)	14 (11.0)	16 (15.3)	2.306
Fish Creek 1976	11 (7.8)	12 (16.4)	9 (8.6)	19 (10.2)	2.554
Fish Creek 1977	12 (10.3)	17 (15.2)	6 (5.6)	16 (20.0)	1.279
Nile Creek	10 (11.0)	20 (18.8)	7 (8.0)	13 (12.2)	1.117
Council Lake	6 (5.5)	13 (13.0)	8 (7.6)	11 (12.0)	0.152
Vernonia Lake	16 (12.3)	14 (15.0)	7 (4.6)	12 (17.2)	4.092*
Mud Lake	17 (14.6)	9 (13.0)	5 (2.9)	19 (19.7)	3.236
Red Top Meadows	9 (9.2)	21 (19.7)	11 (10.6)	9 (10.6)	0.355

* $p<.05$

Table 6. Comparison, by Spearman's rank correlation test, of EST-1 and AO-1 allele frequencies between selected samples of Elaphrus americanus. Results of T-tests (t) for significance (p) of correlations (r) are given. See Table 1 and 2 for allele frequencies.

	EST-1			AO-1		
Sites compared**	r	t 7df	p	r	t 4df	p
P 1976 - P 1977	.829	3.923	.003	.857	3.333	.014
F 1976 - F 1977	.688	2.507	.020	.841	3.105	.017
A 1976 - A 1977	-.034	-0.944	.190*	.754	2.293	.041
R.T. - V.L.	.728	2.810	.013	.886	3.816	.009
R.T. - M.L.		no data		.714	2.041	.055
F 1976 - B.R.	.493	1.502	.087*	.943	5.660	.002
F 1976 - N.C.	.639	2.196	.031	.886	3.816	.009
F 1976 - A.C.	.385	1.103	.154*	.754	2.294	.041
F 1976 - R.T.	.745	2.953	.010	.943	5.659	.002
F 1976 - C.L	.561	1.794	.057	.986	11.662	.000
R.T. - M.H.M.	.456	1.355	.108*	.600	1.500	.104*

* p<<.95 that correlation is significant.
** A = Ashford, A.C. = Anderson Creek, B.R. = Brooklyn Road, C.L. = Council Lake, F = Fish Creek, M.H.M. = Mt. Hood Meadows, M.L. = Mud Lake, N.C. = Nile Creek, P = Paradise, R.T. = Red Top Meadows, V.L. = Vernonia Lake.

Table 7. Relationship of small samples to larger samples of *Elaphrus americanus* from the Pacific Northwest showing the probability of obtaining the actually observed number of individuals with a particular phenotype if that sample had been taken from an adjacent larger sample. Sample size is given in brackets.

	Localities (small samples)					
	Mt. Hood Meadows**** (15)			Mountain Meadows** (12)	Mimulus Lake**** (15)	
AO-1 phenotype	e/e	e/g	g/g	g/g	g/g	
Number observed	3	7	3	7	11	
Localities (large samples)	Probability					
Mud Lake	.122	.008	.129			
Red Top Meadow	.245	.190	.246			
Paradise 1976****	.000	.000	.017	.196	.189	
Paradise 1977****	.000	.000	.000	[illegible]	.225	
Fish Creek 1976						
Fish Creek 1977						
Anderson Creek					.001	
Bagley Lakes****					.120	
EST-1 phenotype					i/i	i/i
Number observed					6	7
Paradise 1977****					.000	.000
Anderson Creek					.004	.000
Bagley Lakes****					.047	.000

** subalpine forest ****subalpine parkland.

Table 8. Comparison, by Spearman's rank correlation test, of the distribution of EST-1 and AO-1 allozymes with respect to colour morphs in selected samples of Elaphrus americanus. Correlations (r) which are positive indicate independence of colour and isozyme variation. Results of T-tests (t) for significance (p) of correlations are given.

Colour forms compared and locality	EST-1				AO-1			
	r	t	df	p	r	t	df	p
Copper - Green Paradise 1976 & 1977	.850	3.677	5	.007	1.000	∞	2	.000
Copper - Green Bagley Lakes	.606	1.865	6	.054	.500	.816	2	.250*
Melanic - Green Red Top Meadows & Council Lake	.737	3.617	10	.002	.753	2.294	4	.041

* p<<.95 that correlation is significant.

Table 9. Colour morph frequencies in samples of Elaphrus americanus from the Pacific Northwest. Number of individuals observed in each class is given in brackets.

Locality	Colour morph				
	Green	Copper (Mt. Rainier)	Copper (Mt. Baker)	Melanic	Others
Mimulus Lake****	.214 (3)	-	.500 (7)	-	.286 (4)
Anderson Creek	1.000 (41)	-	-	-	-
Bagley Lakes****	.521 (25)	-	.299 (11)	-	.250 (12)
Brooklyn Road	.977 (42)	-	-	-	.023 (1)
Ashford 1976	.980 (50)	-	-	-	.020 (1)
Ashford 1977	.975 (39)	-	-	.025 (1)	-
Fish Creek 1977	.904 (47)	-	.058 (3)	-	.038 (2)
Mountain Meadows**	.833 (10)	-	-	.083 (1)	.083 (1)
Paradise 1976****	.540 (27)	.300 (15)	-	-	.160 (8)
Paradise 1977****	.479 (23)	.375 (18)	-	-	.146 (7)
Nile Creek	1.000 (51)	-	-	-	-
Council Lake**	.553 (21)	-	-	.208 (10)	.146 (7)
Vernonia Lake	.840 (42)	-	-	-	.160 (8)
Mud Lake	.451 (23)	.039 (2)	-	.353 (18)	.157 (8)
Red Top Meadows	.200 (11)	-	-	.380 (19)	.400 (20)
Mt. Hood Meadows****	.500 (9)	-	.055 (1)	.444 (8)	-

** subalpine forest ****subalpine parkland - morph not observed.

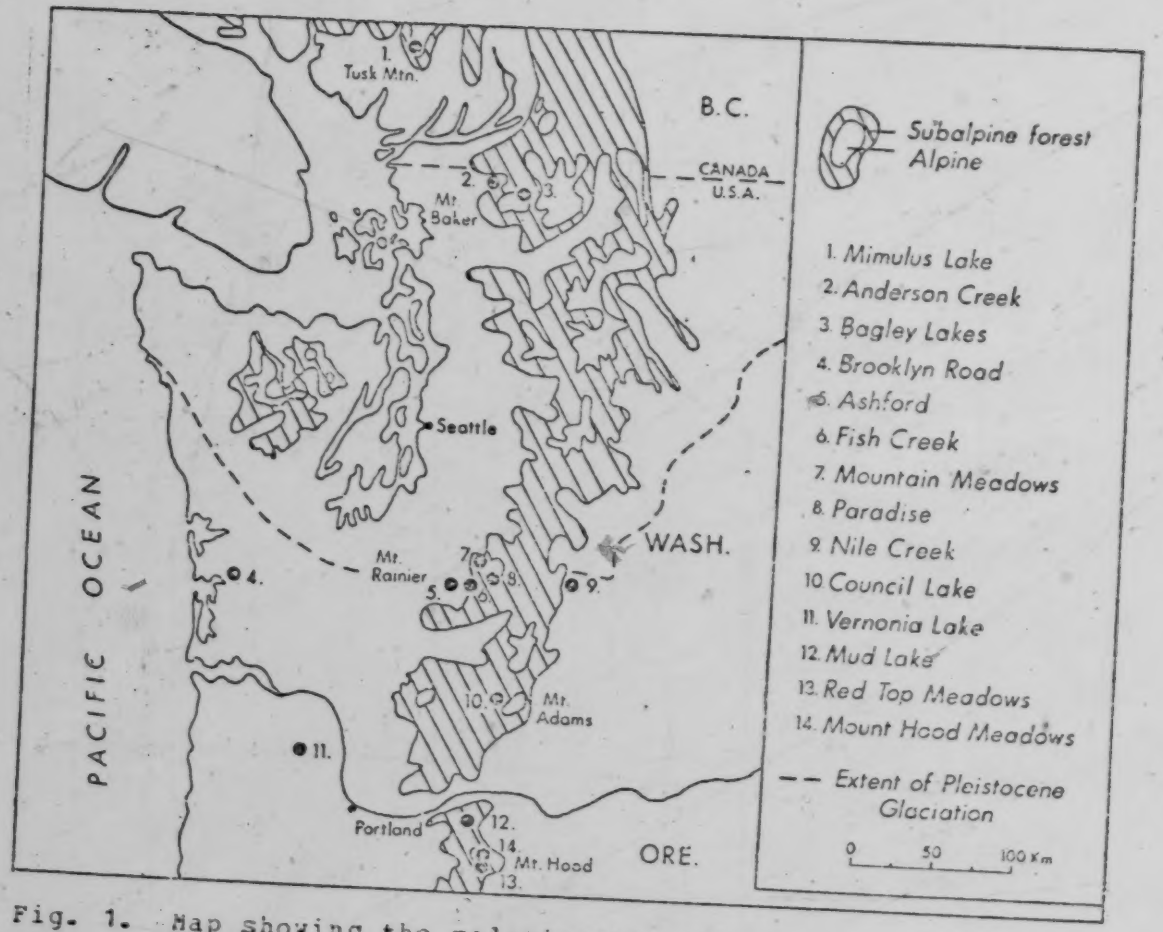

Fig. 1. Map showing the relationship of subalpine forest, alpine areas, limits of Cordilleran glaciation in the Pleistocene and collection sites of Elaphrus americanus. Map compiled from Crandell (1965), Denny (1970) and Franklin and Dryness (1973).

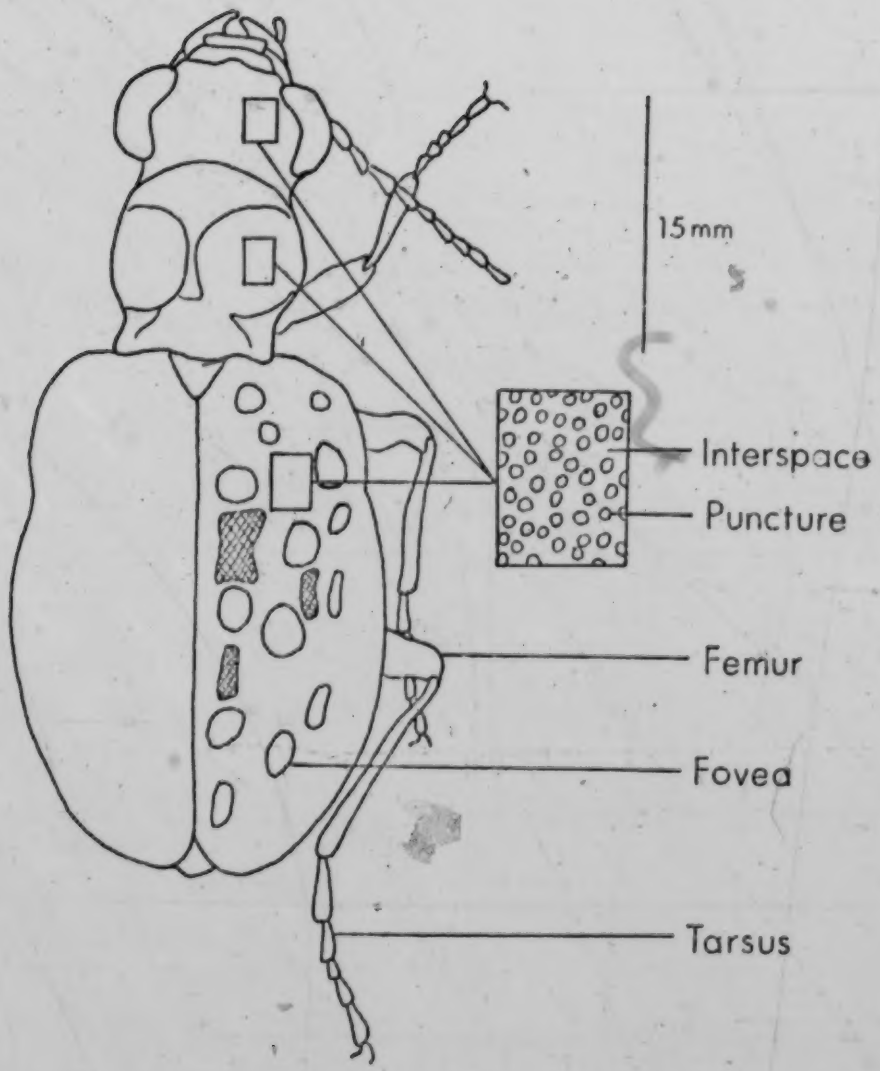

Fig. 2. Dorsal view of an adult male of Elaphrus americanus showing principal features which determine overall body colour. Foveae (depressions) probably disrupt the outline of the body.

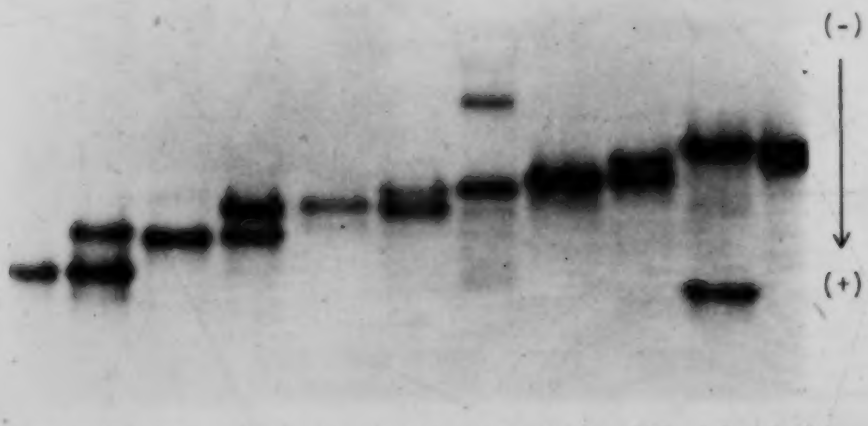

Fig. 3. Polyacrylamide gel showing banding patterns of a monomeric esterase (EST-1) from individuals of Elaphrus americanus from various localities in the Pacific Northwest. Note satellite bands (light grey) below main bands for m/q, k/k, j/j, i/j. The individual scored k/k has a slow-moving 1-napthyl acetate-specific band (grey).

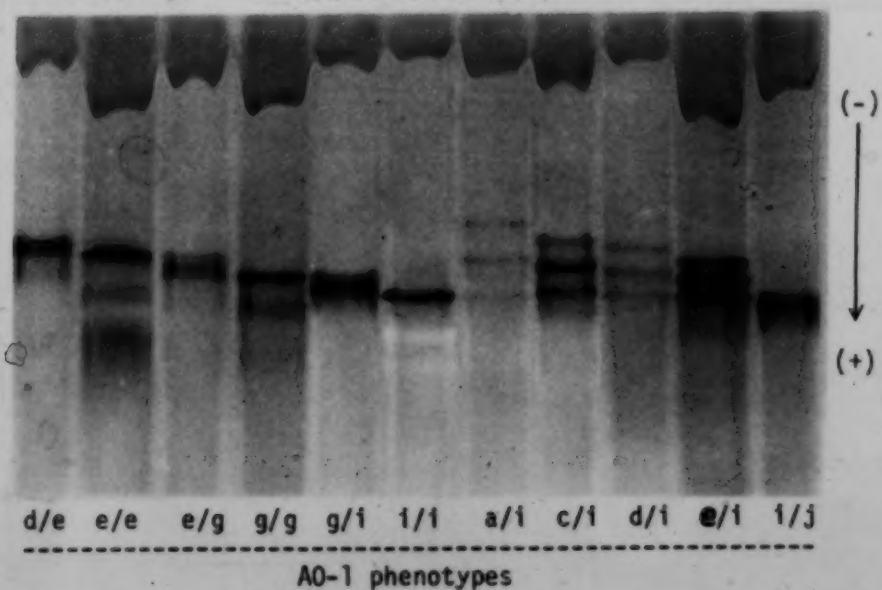

Fig. 4. Polyacrylamide gel showing banding patterns of a dimeric aldehyde oxidase enzyme (AO-1) from individuals of Elaphrus americanus from various localities in the Pacific Northwest.

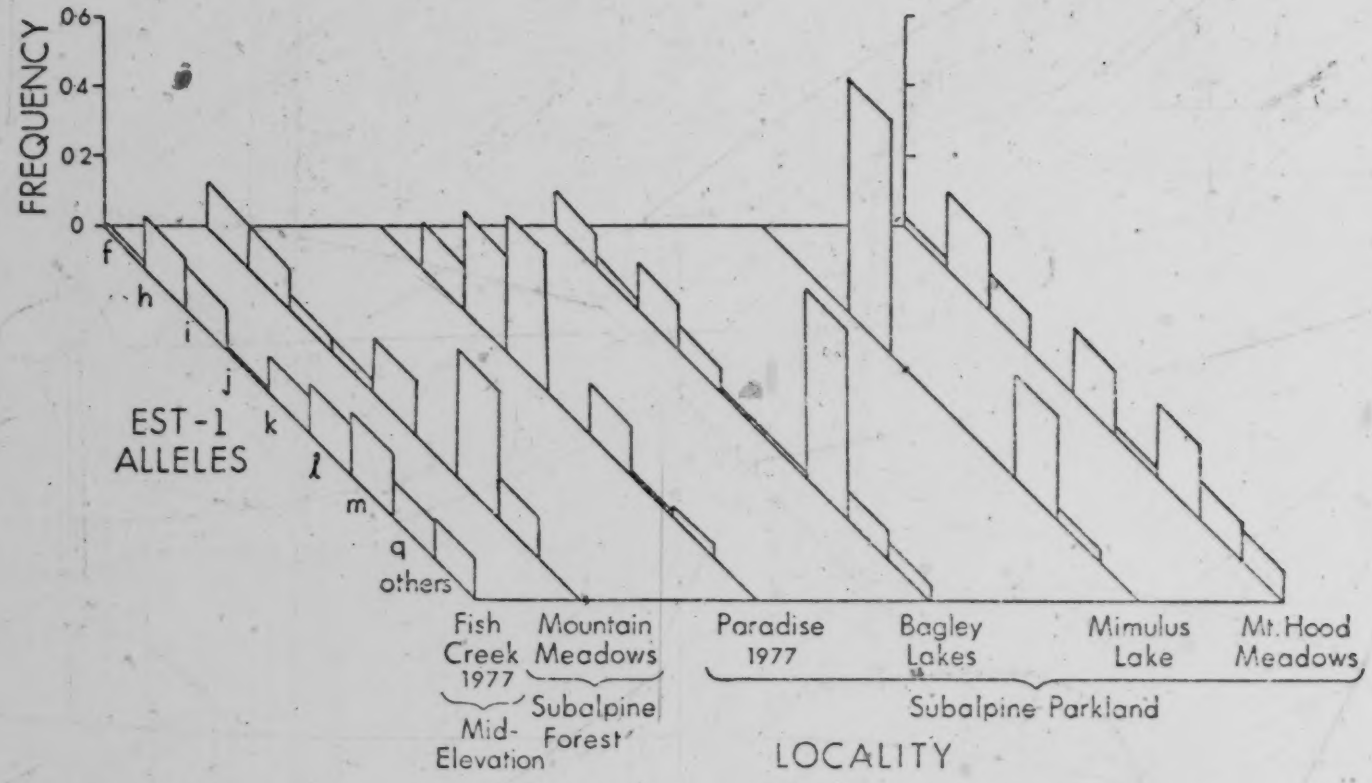

Fig. 5. Frequency distributions of EST-1 alleles from samples of Elaphrus americanus from four mountains (compare with Fig. 1). Mt. Rainier samples are compared from mid-altitude (Fish Creek), subalpine forest (Mountain Meadows) and subalpine parkland (Paradise). Also compared are subalpine parkland samples from Mt. Baker (Bagley Lakes), Tusk Mtn. (Mimulus Lake) and Mt. Hood (Mt. Hood Meadows).

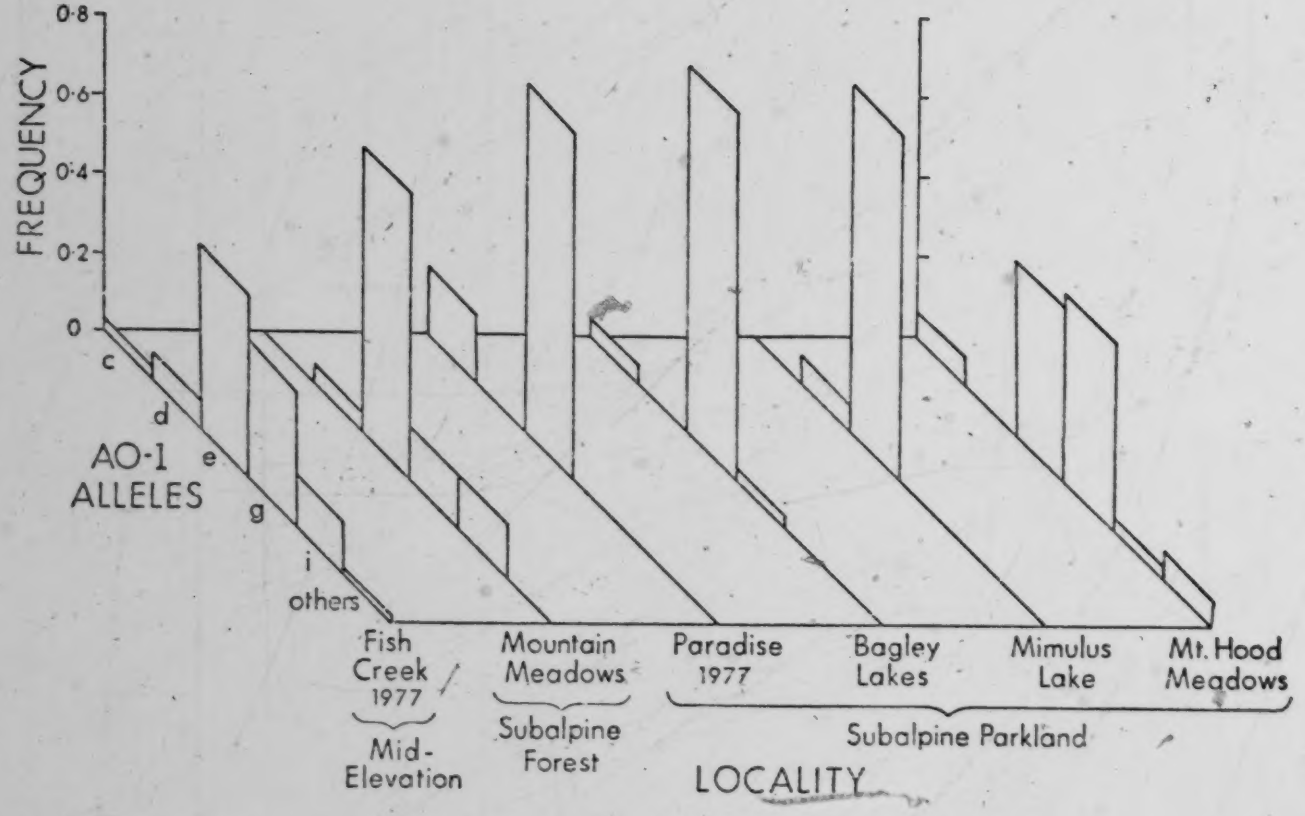

Fig. 6. Frequency distributions of AO-1 alleles from samples of Elaphrus americanus from four mountains (compare with Fig. 1). Mt. Rainier samples are compared from mid-altitude (Fish Creek), subalpine forest (Mountain Meadows) and subalpine parkland (Paradise). Also compared are subalpine parkland samples from Mt. Baker (Bagley Lakes), Tuak Mtn. (Mimulus Lake) and Mt. Hood (Mt. Hood Meadows).